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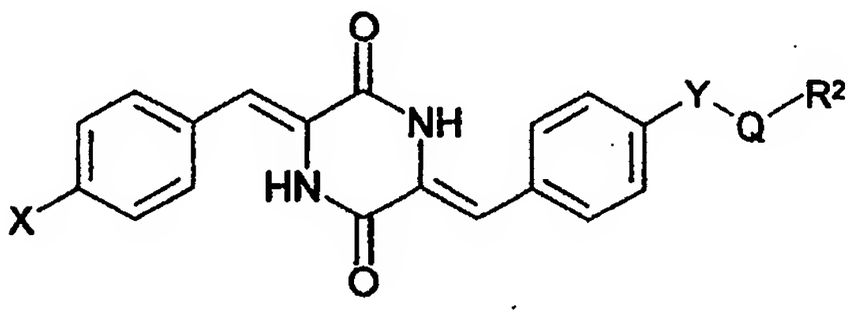
(54) Abstract Title
Diketopiperazines

(57) Compounds which are diketopiperazines of formula (I):



I

wherein X is selected from H and NHCOZ wherein Z is a thiophene or furan ring;
Y is selected from O, S, NHCO, and CONH;
Q is a C_1-C_8 alkylene chain which is unsubstituted or substituted at any position along the chain by a group CO_2R^1 where R^1 is H or C_1-C_6 alkyl; and
R^2 is selected from
a) CO_2R^1 wherein R^1 is as defined above;
b) tetrazole, which is bonded to Q via its carbon atom;
c) SO_3H;
and
d) $CONHR^3$ where NHR^3 derives from an amino acid R_3NH_2;
and the pharmaceutically acceptable salts thereof have activity as inhibitor; of plasminogen activator inhibitor PAI-1 and may be used in treating haemostatic or thrombotic disorders.

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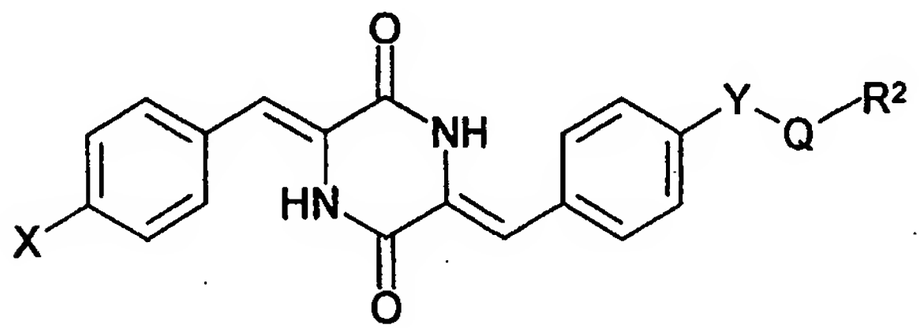
PHARMACEUTICAL COMPOUNDS

The present invention relates to compounds useful as inhibitors of plasminogen activator inhibitor (PAI-1), to their preparation and to pharmaceutical and veterinary compositions containing them.

Plasminogen activators (PAs) are serine proteases which control the activation of the zymogen, plasminogen, to the active enzyme plasmin. Plasmin is important in a variety of physiological and pathophysiological processes including fibrinolysis, atheroma, cancer, inflammation, wound healing and angiogenesis. Plasminogen is converted to proteolytically active plasmin by either tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (Vassalli *et al*; J.Clin.Invest. 88, 1067-1072, 1991). Plasminogen activator inhibitor (PAI-1) is the major physiological inhibitor of plasminogen activators, and an increase in the level of PAI-1 has been proposed as a risk factor in thrombotic disease (Dawson *et al*, Atherosclerosis; 95, 105-117, 1992), and is associated with a poor prognosis in a variety of cancers (Pappot *et al*, Biol.Chem.Hoppe-Seyler; 376, 259-267, 1995). Inhibition of PAI-1 may therefore be of benefit in thromboembolic disease, atherosclerosis, inflammatory disease, tumour invasion, metastasis and tumour angiogenesis (Exp. Opin. Invest. Drugs 1997, 6(5), 539-554 (Charlton) and Nature Medicine vol 4, No. 8, 923-928, 1998 (Bajou *et al*).

The present invention provides a compound which is a diketopiperazine of formula (I)



I

wherein X is selected from H and NHCOZ wherein Z is a thiophene or furan ring;

Y is selected from O, S, NHCO and CONH;

Q is a C_1-C_8 alkylene chain which is unsubstituted or substituted at any position along the chain by a group CO_2R^1 where R^1 is H or C_1-C_6 alkyl; and

R^2 is selected from:

a) CO_2R^1 wherein R^1 is as defined above;

b) tetrazole, which is bonded to Q via its carbon atom;

c) SO_3H;

and

d) $CONHR^3$ where NHR^3 derives from an amino acid R_3NH_2;

or a pharmaceutically acceptable salt thereof.

In one embodiment of the invention the diketopiperazine is of formula (I) as defined above wherein:

X is NHCOZ wherein Z is thiophene;

Y is selected from O, NHCO and CONH;

Q is a C_3-C_8 alkylene chain which is unsubstituted or substituted at any position by a group CO_2R^1 as defined above; and

R^2 is (a) CO_2R^1 as defined above, (b) tetrazole bonded to Q via its carbon atom, or (c) $CONHR^3$ wherein R^3 is $-CH(COOH)CH_2CH_2COOH$.

In a preferred embodiment of formula (I)

X is NHCOZ wherein Z is thiophen-2-yl;

Y is O or NHCO;

Q is C_3-C_8 alkylene which is unsubstituted or substituted at the carbon atom adjacent to Y by a group CO_2R^1 as defined above; and

R^2 is CO_2R^1 as defined above, or is tetrazole.

In general it is preferred for R^2 in formula (I) to be COOH or a tetrazole ring. It is generally preferred for X to be NHCOZ wherein Z is thiophen-2-yl. It is also generally preferred for Q to be a C_6-C_8 alkylene chain. When R^1 is alkyl it is preferably t-butyl or methyl such that the group $COOR^1$ is a t-butyl ester or methyl ester group.

A C_1-C_8 alkylene chain is $-(CH_2)_n-$ wherein n is from 1 to 8. Typically n is from 3 to 8. Examples of C_1-C_8 alkylene include methylene (n=1), ethylene (n=2), propylene (n=3), butylene (n=4), pentylene (n=5), hexylene (n=6), heptylene (n=7) and octylene (n=8).

A C_1-C_6 alkyl group is branched or unbranched. It is, for instance, C_1-C_4 alkyl. Examples include methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl and t-butyl.

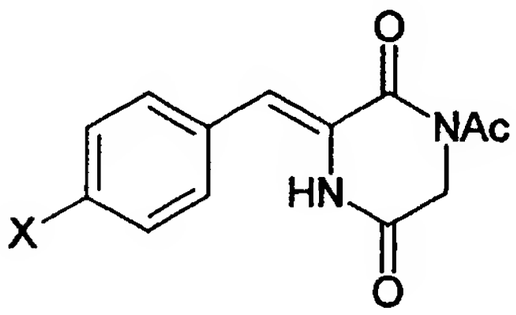
When Z is a thiophene or furan ring, it may be attached to the carbonyl moiety of the NHCO group at any ring position. Typically it is attached at ring position 2, such that Z is thiophen-2-yl or furan-2-yl.

Examples of preferred compounds of the invention are shown in the table below:

XR Number	Chemical Name
1	2-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-pentanedioic acid di-*tert*-butyl ester
2	2-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-pentanedioic acid
3	5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoic acid methyl ester
4	5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoic acid
5	4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-butyric acid methyl ester
6	4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-butyric acid
7	7-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid methyl ester
8	9-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-nonanoic acid methyl ester
9	7-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid
10	9-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-nonanoic acid
11	2-{5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoylamino}-pentanedioic acid di-*tert*-butyl ester
12	2-{5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoylamino}-pentanedioic acid
13	7-[4-(5-Benzylidene-3,6-dioxo-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid methyl ester
14	7-[4-(5-Benzylidene-3,6-dioxo-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid
15	5-[4-(5-{4-[(Furan-2-carbonyl)-amino]-benzylidene}-3,6-dioxo-piperazin-2-

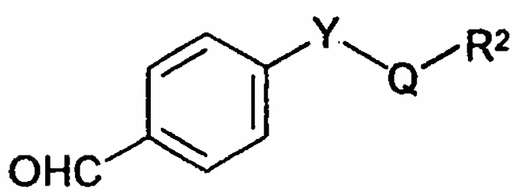
	ylidenemethyl)-phenoxy]-pentanoic acid methyl ester
16	4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-butane-1-sulfonic acid
17	5-[4-(5-{4-[(Furan-2-carbonyl)-amino]-benzylidene}-3,6-dioxo-piperazin-2-ylidenemethyl)-phenoxy]pentanoic acid
18	8-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-octanoic acid methyl ester
19	8-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-octanoic acid
20	Thiophene-2-carboxylic acid [4-(3,6-dioxo-5-{4-[7-(1*H*-tetrazol-5-yl)-heptyloxy]-benzylidene}-piperazin-2-ylidenemethyl)-phenyl]-amide
21	3-Benzylidene-6-{4-[7-(1*H*-tetrazol-5-yl)-heptyloxy]-benzylidene}-piperazine-2,5-dione

Compounds of formula (I) may be prepared by a process which comprises condensing a compound of Formula (II):



(II)

wherein X is as defined above, with a compound of formula (III):

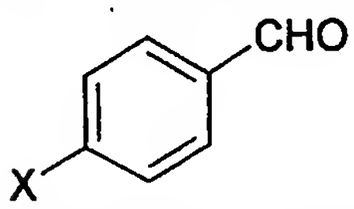


(III)

wherein Y, Q and R^2 are as defined above, in the presence of a base in an organic solvent.

Preferably the solvent is a polar aprotic solvent, for instance dimethylformamide (DMF) or tetrahydrofuran (THF). The base is typically a group I or group II metal carbonate, for instance caesium carbonate.

Compounds of Formula (II) are prepared by condensing 1,4-diacetyl-2,5-piperazinedione with a compound of formula (IV):



(IV)

wherein X is as defined above, in the presence of a base in an organic solvent.

Any suitable solvent or base may be used. Typically the solvent is a polar aprotic solvent, for instance DMF or THF. The base may be, for instance potassium t-butoxide and the solvent may be, for instance, THF.

Compounds of Formula (IV) are prepared by standard experimental techniques which would be familiar to a person skilled in the art. Some of these are described in the Reference Examples which follow.

If desired one compound of Formula (I) may be converted into another compound of Formula (I). For example, an alkyl ester, for example a methyl or ethyl ester, may be converted into the corresponding carboxylic acid by acid or alkaline hydrolysis. In another example a compound of formula (I) containing an ester group, for instance a *tert*-butyl ester, may be converted into the corresponding carboxylic acid by treatment with trifluoroacetic acid.

A diketopiperazine of formula (I) may be converted into a pharmaceutically acceptable salt, and a salt may be converted into the free diketopiperazine, by conventional methods. Salts may be formed by compounds containing a carboxylic acid or tetrazole function. Suitable salts include those with pharmaceutically acceptable inorganic bases. Examples of such bases include ammonia and carbonates, hydrogencarbonates and hydroxides of group I and group II metals such as sodium, potassium, magnesium and calcium.

The diketopiperazines of formula (I) and their pharmaceutically acceptable salts and esters (referred to hereinafter as the "present compounds") have utility as inhibitors of PAI-1. They may thus be used to treat a disease or disorder associated with elevated or inappropriate levels of PAI-1.

Elevated levels of PAI-1, by reducing the net endogenous fibrinolytic capacity, can contribute to the pathogenesis of various thrombotic disorders including coronary artery disease, acute myocardial infarction, unstable angina, deep vein thrombosis and recurrent venous thromboembolism (Declerck *et al*; J.Intern.Med. 236,425-432, 1994. Aznar *et al*; Haemostasis 24,243-251,1994. Gray et al;

Haemostasis, 73, 261-267, 1995). The present compounds therefore can act as inhibitors of the tPA/PAI-1 interaction. The present compounds can be used in the treatment of haemostatic disorders. A human or animal, e.g. a mammal, can therefore be treated by a method comprising administration of a therapeutically effective amount of a diketopiperazine of formula (I) or a pharmaceutically or veterinarily acceptable salt thereof.

Tissue plasminogen activator (tPA) is used as a thrombolytic agent in the treatment of thrombotic disorders. The efficacy of the tPA in this role may be enhanced if it is administered together with a PAI-1 inhibitor. A human or animal, e.g. a mammal, can therefore be treated by a method comprising the combined administration of a therapeutically effective amount of tPA and a therapeutically effective amount of any one of the present compounds. The present invention also provides products containing a diketopiperazine of formula (I) or a pharmaceutically acceptable salt or ester thereof and tPA as a combined preparation for simultaneous, separate or sequential use in the treatment of thrombotic disorders, for example where there is inappropriate PAI-1 activity. In such products the present compound is formulated for oral or parenteral (intravenous, intramuscular or subcutaneous) administration and the tPA is formulated for intravenous administration.

As one example, during acute myocardial infarction (MI) one of the present compounds may be administered to a patient together with tPA to enhance the efficacy of the tPA treatment. As a further example, early re-occlusion following treatment of a patient with tPA may be prevented by the post-MI administration of one of the present compounds.

The present compounds have been tested in a PAI-1 functional assay. In this assay, a compound is incubated with PAI-1 prior to addition to the tPA assay system. Inhibition of PAI-1 by compounds of Formula (I) results in the production of plasmin from plasminogen. In turn, plasmin cleaves the chromogenic substrate S2251 (Kabi Vitrum) producing pNA (p-nitroaniline) which is detected spectrophotometrically at 405 nm (K.Nilsson et al, Fibrinolysis (1987) 1, 163-168). The results of the assay are reported below.

A selection of the present compounds has also been tested in a clot lysis assay which can quantify the PAI-1 inhibitory activity of the compounds by measuring the rate of fibrinolysis (Ehnebom J, Kristianssen C, Bjorquist P, Deinum J,

Bostrum S. Thrombosis and Haemostasis, 1993, 69, 1330a). This is referred to as the fibrin plate assay and is described in more detail in Example 3 which follows

The present compounds may be administered in a variety of dosage forms, for example orally such as in the form of tablets, capsules, sugar- or film-coated tablets, liquid solutions or suspensions or parenterally, for example intramuscularly, intravenously or subcutaneously. The present compounds may therefore be given by injection or infusion.

The dosage depends on a variety of factors including the age, weight and condition of the patient and the route of administration. Typically, however, the dosage adopted for each route of administration when a compound of the invention is administered alone to adult humans is 0.001 to 10 mg/kg, most commonly in the range of 0.01 to 5 mg/kg, body weight. Such a dosage may be given, for example, from 1 to 5 times daily by bolus infusion, infusion over several hours and/or repeated administration.

When one of the present compounds is administered in combination with tPA to adult humans, the dosage adopted for each route of administration is typically from 0.001 to 10 mg, more typically 0.01 to 5 mg per kg body weight for a compound of the invention and from 5 to 500 mg administered intravenously for the tPA. A suitable dosage regimen for the tPA is 100 mg given intravenously over 3 hours as follows: 10% of the total dose as an i.v. bolus over 1-2 minutes, 50% of the total dose as an infusion over 1 hour, 40% of the total dose as an infusion over the subsequent 2 hours.

A diketopiperazine of formula (I) or a pharmaceutically acceptable salt or ester thereof is formulated for use as a pharmaceutical or veterinary composition also comprising a pharmaceutically or veterinarily acceptable carrier or diluent. The compositions are typically prepared following conventional methods and are administered in a pharmaceutically or veterinarily suitable form. An agent for use as an inhibitor of PAI-1 comprising any one of the present compounds is therefore provided.

For example, the solid oral forms may contain, together with the active compound, diluents such as lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants such as silica, talc, stearic acid, magnesium or calcium stearate and/or polyethylene glycols; binding agents such as starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose, or polyvinyl pyrrolidone;

disintegrating agents such as starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs, sweeteners; wetting agents such as lecithin, polysorbates, lauryl sulphates. Such preparations may be manufactured in known manners, for example by means of mixing, granulating, tabletting, sugar coating, or film-coating processes.

Liquid dispersions for oral administration may be syrups, emulsions and suspensions. The syrups may contain as carrier, for example, saccharose or saccharose with glycerol and/or mannitol and/or sorbitol. In particular, a syrup for diabetic patients can contain as carriers only products, for example sorbitol, which do not metabolise to glucose or which only metabolise a very small amount to glucose. The suspensions and the emulsions may contain as carrier, for example, a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose or polyvinyl alcohol.

Suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier such as sterile water, olive oil, ethyl oleate, glycols such as propylene glycol, and, if desired, a suitable amount of lidocaine hydrochloride. Some of the present compounds are insoluble in water. Such compounds may be encapsulated within liposomes.

The present invention will be further illustrated in the following Examples:

Reference Example 1: Preparation of Compounds of General Formula (IV)

Thiophene-2-carboxylic acid (4-formyl-phenyl)-amide

4-Nitrobenzaldehyde (16.65g) was treated with ethylene glycol (12.29mL) and p-toluenesulphonic acid (catalytic) in refluxing toluene (170mL) in a Dean and Stark apparatus to yield the corresponding acetal (20.43g). The nitro group was then reduced to the amine by catalytic hydrogenation with PtO_2 in dry tetrahydrofuran using the Parr apparatus to yield 4-[1,3]dioxolan-2-yl-phenylamine. This material (51.2mmol) was reacted with 2-thiophenecarbonyl chloride (5.48mL) in dry tetrahydrofuran (200mL) in the presence of triethylamine (7.14mL). Once the reaction was complete, hydrochloric acid (2N) was added and the mixture stirred for 30mins. The organics were then extracted into ethyl acetate, washed with water, dried ($MgSO_4$ or Na_2SO_4), reduced *in vacuo*, and trituration with ether yielded the title compound (11.71g).

Other compounds of general formula (IV) may be prepared using an analogous route to above using the appropriate activated acid such as an acid chloride, e.g. 2-furancarbonyl chloride yields furan-2-carboxylic acid (4-formyl-phenyl)-amide.

Reference Example 2: Preparation of Compounds of General Formula (II)

Thiophene-2-carboxylic acid [4-(4-acetyl-3,6-dioxo-piperazin-2-ylidenemethyl)-phenyl]-amide

To a solution of 1,4-diacetyl-2,5-piperazinedione (0.85g, 1 equivalent) (for synthesis see S.M. Marcuccio and J.A. Elix, *Aust. J Chem* ,1984, 37, 1791-4) and thiophene-2-carboxylic acid (4-formyl-phenyl)-amide (0.99g,1 equivalent) in dry tetrahydrofuran (10mL) cooled to 0°C, was added a solution of potassium *t*-butoxide (0.53g,1.1 equivalents) in *t*-butanol (10mL) . The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was then diluted with ethyl acetate, washed with water, and the solid which persisted was collected by filtration and washed with ether to yield the title compound (0.72g, 45%).

The following compounds of general formula (II) may be prepared in an analogous manner using the appropriate aldehyde;

1-Acetyl-3-benzylidene-piperazine-2,5-dione;

Furan-2-carboxylic acid [4-(4-acetyl-3,6-dioxo-piperazin-2-ylidenemethyl)-phenyl]-amide.

Reference Example 3: Preparation of Compounds of General Formula (III)

Reference Example 3A: 8-(4-Formyl-phenoxy)-octanoic acid methyl ester

To a stirring suspension of 8-bromooctanoic acid (4.0g) and trimethyloxonium tetrafluoroborate (2.92g) in dichloromethane (100mL) was added diisopropylethyl amine (3.44mL). After stirring for 18 hours and aqueous work-up, 8-bromooctanoic acid methyl ester was isolated as a yellow liquid (4.15g, 97%).

To a stirring solution of 4-hydroxybenzaldehyde (1.03g) in anhydrous N,N-dimethylformamide (25mL) was added sodium hydride(60% dispersion in mineral oil, 324mg). Once effervescence had ceased, 8-bromooctanoic acid methyl ester (2.0g) was added and the reaction mixture was stirred for 48 hours at room temperature. The reaction mixture was poured onto hydrochloric acid (2N), extracted into ethyl acetate,

dried ($MgSO_4$) and the solvent removed *in vacuo*. Trituration of the residue with hexane yielded the title compound as a yellow solid (848mg).

In an analogous manner other aldehydes may be prepared using the appropriate haloalkyl ester.

For example:

i) 5-(4-Formyl-phenoxy)-pentanoic acid methyl ester may be prepared using methyl 5-bromovalerate (yield=78%).

ii) 2-[5-(4-Formyl-phenoxy)-pentanoylamino]-pentanedioic acid di-*tert*-butyl ester may be prepared using 2-(5-bromo-pentanoylamino)-pentanedioic acid di-*tert*-butyl ester and using potassium carbonate as the base (yield=24%)

2-(5-Bromo-pentanoylamino)-pentanedioic acid di-*tert*-butyl ester may in turn be prepared by treating di-t-butyl glutamate hydrochloride with bromovaleryl chloride in dichloromethane in the presence of pyridine (2 equivalents).

Reference Example 3B: 4-(4-Formyl-phenoxy)-butane-1-sulfonic acid, sodium salt

A mixture of 4-hydroxybenzaldehyde (5g), 1-bromo-4-chlorobutane (7.0g) and potassium carbonate (6.0g) in anhydrous N,N-dimethylformamide (50mL) was stirred at 60°C for 18 hours. Aqueous work-up yielded 4-(4-chloro-butoxy)-benzaldehyde (yield=92%).

A mixture of 4-(4-chloro-butoxy)-benzaldehyde (2.0g) and sodium sulphite (3.0g) in an ethanol/water mixture (14mL/6mL) was heated to reflux for six hours. The solvents were removed *in vacuo* and the residues were then dissolved in water and washed with hexane. The aqueous phase was then reduced *in vacuo* and the residue recrystallised twice from ethanol to yield the title compound as a white solid (0.69g).

Reference Example 3C: 7-(4-Formyl-phenylcarbamoyl)-heptanoic acid methyl ester

4-Nitrobenzaldehyde was converted to the acetal and then reduced to yield 4-[1,3]dioxolan-2-yl-phenylamine as described in Reference Example 1.

To a solution of freshly prepared 4-[1,3]dioxolan-2-yl-phenylamine (0.014mol) in tetrahydrofuran (60mL) cooled to 0°C was added triethylamine (1 equivalent) and methyl suberyl chloride(2.85g). The reaction mixture was stirred for 18 hours before hydrochloric acid (2N) was added. The organics were extracted into ethyl acetate,

dried, and the solvent removed *in vacuo* to yield the title compound as an off-white solid (3.18g, 79%).

Other aldehydes may be prepared in an analogous manner to Example 3C using the appropriate acid chloride.

For example 9-(4-formyl-phenylcarbamoyl)-nonanoic acid methyl ester may be prepared using methyl sebacoyl chloride (91%).

Reference Example 3D: 4-(4-Formyl-benzoylamino)-butyric acid methyl ester

A mixture of 4-carboxybenzaldehyde (5g), thionyl chloride (10mL), and N,N-dimethylformamide (catalytic) in toluene (25mL)was heated to reflux for one hour by which time the reaction mixture had become a clear solution. The solvent was removed *in vacuo* to yield an oil. Hexane was added and after standing 4-formyl-benzoyl chloride was collected as a cream solid (3.85g, 68%).

To a stirring suspension of methyl-4-aminobutyrate hydrochloride (729mg) in dichloromethane (50mL) at 0°C was added triethylamine (1.32mL) and 4-formyl-benzoyl chloride (1g). After stirring for 16 hours, aqueous work-up yielded crude material, which was purified using flash chromatography and then triturated from ether to yield the title compound as a white solid (721mg,61%).

Other aldehydes may be prepared in an analogous manner using the appropriate amine. For example 2-(4-formyl-benzoylamino)-pentanedioic acid di-*tert*-butyl ester may be prepared from 2-amino-pentanedioic acid di-*tert*-butyl ester hydrochloride (94%).

Reference Example 3E 4-[7-(1*H*-Tetrazol-5-yl)-heptyloxy]-benzaldehyde

A mixture of 4-hydroxybenzaldehyde (5.00g, 40.94mmol), potassium carbonate (14.15g, 2.5 equivalents) and 1,7-dibromoheptane (26.5g, 2.5 equivalents) was heated to 50°C in DMF for 4 hours. The reaction mixture was then cooled, diluted with ethyl acetate, washed with water, dried ($MgSO_4$) and the solvent removed *in vacuo* to yield an oil, which was purified using flash chromatography (hexane/ethyl acetate 4:1) to give 4-(7-bromo-heptyloxy)-benzaldehyde as a white solid (7.30g, 60%).

A mixture of 4-(7-Bromo-heptyloxy)-benzaldehyde (1.00g, 3.34mmol) and potassium cyanide (0.26g, 1.2 equivalents) was heated to reflux in ethanol (10mL)

and water (3mL) for 3 hours. The reaction mixture was then cooled, diluted with ethyl acetate (100mL), washed with brine (2 times), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield 8-(4-formyl-phenoxy)-octanenitrile as a pale oil (88%).

A mixture of 8-(4-formyl-phenoxy)-octanenitrile (506mg, 2.07mmol), ethylene glycol (231µL, 2 equivalents), pTSA (catalytic) and toluene (5mL) was heated to reflux in a flask attached to a Dean-Stark apparatus. After 2 hours the reaction mixture was cooled, diluted with ethyl acetate (100mL), washed with sodium bicarbonate solution, dried ($MgSO_4$) and the solvent removed *in vacuo* to yield 8-(4-[1,3]dioxolan-2-yl-phenoxy)-octanenitrile as a pale oil (81%).

A mixture of 8-(4-[1,3]dioxolan-2-yl-phenoxy)-octanenitrile (1.05g, 3.63mmol), sodium azide (1.18g, 5 equivalents), ammonium chloride (199mg, 1.05equivalents) and triethylammonium chloride (500mg, 1 equivalent) in dry N,N-dimethylformamide (7ml) was heated to 120°C for 18 hours. The reaction mixture was cooled, diluted with ethyl acetate (100mL), extracted with NaOH solution (2M), acidified (HCl, 2M) and then re-extracted into ethyl acetate (3X). The organic extractions were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to yield a yellow solid. This was purified using flash chromatography (silica, ethyl acetate) to yield the title compound as an off-white solid (11%).

Example 1: Preparation of Compounds of General Formula(I)

8-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-octanoic acid methyl ester

A mixture of thiophene-2-carboxylic acid [4-(4-acetyl-3,6-dioxo-piperazin-2-ylidenemethyl)-phenyl]-amide (1.32g), 8-(4-formyl-phenoxy)-octanoic acid methyl ester (1g), and cesium carbonate (1.16g) in N,N-dimethylformamide (50mL) was heated to 60°C for 2 hours. After cooling, water was added yielding a solid which was collected by filtration and recrystallised from N,N-dimethylformamide to yield the title compound as a yellow solid (168mg)

The following compounds were prepared in an analogous manner using the appropriate starting compounds:

2-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-pentanedioic acid di-*tert*-butyl ester;

5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoic acid methyl ester;
4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-butyric acid methyl ester;
7-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid methyl ester;
9-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-nonanoic acid methyl ester;
2-{5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoylamino}-pentanedioic acid di-*tert*-butyl ester;
7-[4-(5-Benzylidene-3,6-dioxo-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid methyl ester;
5-[4-(5-{4-[(Furan-2-carbonyl)-amino]-benzylidene}-3,6-dioxo-piperazin-2-ylidenemethyl)-phenoxy]-pentanoic acid methyl ester;
4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-butane-1-sulfonic acid (hydrochloric acid,2N, added as part off work-up);
Thiophene-2-carboxylic acid [4-(3,6-dioxo-5-{4-[7-(1*H*-tetrazol-5-yl)-heptyloxy]-benzylidene}-piperazin-2-ylidenemethyl)-phenyl]-amide;
3-Benzylidene-6-{4-[7-(1*H*-tetrazol-5-yl)-heptyloxy]-benzylidene}-piperazine-2,5-dione.

Example 2: Conversion of one Compound of General Formula (I) into another Compound of General Formula (I)

Example 2A: 8-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-octanoic acid

8-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-octanoic acid methyl ester (150mg) was stirred in a mixture of sodium hydroxide solution(2N, 5mL), methanol (5mL) and tetrahydrofuran (5mL) for 30 minutes. The organic solvents were then removed *in vacuo*. Concentrated hydrochloric acid was added to the aqueous residue resulting in the formation of a solid which was collected by filtration and recrystallised from N,N-dimethylformamide to yield the title compound as a yellow solid (21mg,14%).

The following compounds were prepared in an analogous manner starting with the appropriate ester.

5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoic acid;

4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-butyric acid;

7-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid;

9-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-nonanoic acid;

7-[4-(5-Benzylidene-3,6-dioxo-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid;

5-[4-(5-{4-[(Furan-2-carbonyl)-amino]-benzylidene}-3,6-dioxo-piperazin-2-ylidenemethyl)-phenoxy]pentanoic acid.

Example 2B: 2-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-pentanedioic acid

2-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-pentanedioic acid di-*tert*-butyl ester was dissolved in trifluoroacetic acid at 0°C for 1 hour. The resulting solid was collected by filtration, and recrystallised from N,N-dimethylformamide with a few drops of water to yield the title compound as a yellow solid (72%).

Other compounds of general formula (I) may be prepared in a similar manner using the appropriate *tert*-butyl ester starting material. For example 2-{5-[4-(3,6-dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoylamino}-pentanedioic acid was prepared from 2-{5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoylamino}-pentanedioic acid di-*tert*-butyl ester .

Example 3: Testing of the present compounds as PAI-1 inhibitors

The present compounds were tested in a PAI-1 chromogenic substrate assay. In the assay (K.Nilsson, Fibrinolysis (1987) 1, 163-168) each compound was incubated with PAI-1 prior to addition to the tPA assay system. Inhibition of PAI-1

by the compounds of formula (I) resulted in the production of plasmin from plasminogen. In turn, the plasmin cleaved the chromogenic substrate S2251 (Kabi-Vitrum) producing pNA (p-nitroaniline) which was detected spectrophotometrically at 405 nm. This is referred to as the chromogenic assay.

Some of the present compounds were also tested in a clot lysis assay that can quantify the PAI-1 inhibitory activity of the compounds by measuring the rate of fibrinolysis (Ehnebom J, Kristianssen C, Bjorquist P, Deinum J, Bostrum S. Thrombosis and Haemostasis, 1993, 69, 1330a). This is referred to as the fibrin plate assay. In this assay fibrinogen was clotted with thrombin and calcium to give an opaque, insoluble fibrin gel. The gel or clot contained added plasminogen and tissue plasminogen activator (tPA), tPA converted plasminogen into plasmin, and plasmin was then able to degrade the fibrin clot to form soluble fibrin fragments. This process was monitored by measuring absorbance. The addition of PAI-1 to the reaction inhibited tPA cleavage of plasminogen to plasmin and therefore inhibited clot lysis. Preincubation of the present compounds with PAI-1 inhibited PAI-1 activity thus allowing tPA to cleave plasminogen to plasmin and lyse the fibrin clot.

The IC_{50} values for each compound are presented in the tables below. The biological activities of the compounds were in the range 0.1 - 7μM. For example 8-[4-(3,6-dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-octanoic acid had an IC_{50} of 0.2μM in the chromogenic assay.

Chromogenic assay	
Compound No.	**IC_{50}(μM)**
1	0.92
4	1.75
5	3.41
6	6.75
9	0.52
10	0.39
12	2.1
14	3.6

16	1.63
17	5
18	1.93
19	0.20

A comparison of the activity of 19 and 20 in the fibrin plate assay demonstrated the similar activities obtained with R^2 as either a carboxylic acid group or as a tetrazole group.

Fibrin plate assay	
Compound No.	**$IC_{50}(\mu M)$**
19	0.26
20	0.13

Example 4: Characterisation of the present compounds

The compounds prepared in the preceding Examples were characterised by proton N.M.R spectroscopy and mass spectroscopy. All proton N.M.R were performed at 400MHz. Characterisation by mass spectroscopy was performed using desorption chemical ionisation or electrospray ionisation. The results are set out in Table 2:

Table 2

Compound No.	**Molecular formula**	**Mass spec. data**	**^{1}H NMR data**
1	C37H40N4O8S	DCI + NH3 MH+(701,75%) base peak (589)	d6-DMSO 1.40(9H,s); 1.42(9H,s); 1.88-2.12(2H,m); 2.34(2H,t,J=7.4Hz); 4.31-4.39(1H,m); 6.77(1H,s); 6.80(1H,s); 7.21-7.24(1H,m); 7.57(2H,d,J=8.7Hz); 7.65(2H,d,J=8.2Hz); 7.80(2H,d,J=8.7Hz); 7.87(1H,d,J=4.8Hz); 7.92(2H,d,J=8.2Hz); 8.04(1H,d,J=3.4Hz); 8.62(1H,d,J=7.5Hz); 10.35(3H, broad).
2	C29H24N4O8S	ESI m/e MH+ (589,100)	d6-dmso 2.00-2.03(1H,broad m); 2.10-2.12(1H,broad m); 2.38-2.39(2H,m); 4.42-4.44(1H,broad m); 6.78(1H,s); 6.82(1H,s); 7.21-7.24(1H,m); 7.58(2H,d,J=8.2Hz); 7.64(2H,d,J=7.9Hz); 7.80(2H,d,J=8.2Hz); 7.88(1H,d,J=4.5Hz); 7.93(2H,d,J=7.9Hz);

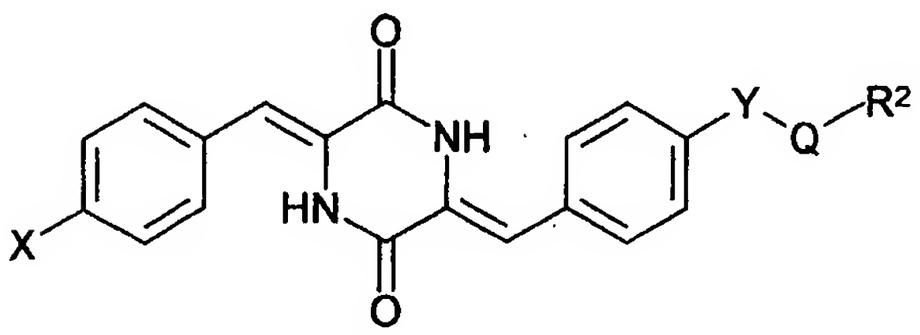
			8.06(1H,d,J=3.3Hz); 8.62(1H,d,J=7.2Hz); 10.35(2H,broad s); 10.40(1H,broad); 12.40(2H,broad peak).
3	C29H27N3O6 S	DCI + CH4 MH+ m/e 546(100)	d6-DMSO 1.62-1.80(4H,m); 2.40(2H,t, J= 7.1Hz); 3.60(3H,s); 4.04(2H,t,J=5.9 Hz); 6.74(1H,s);6.74(1H,s); 6.97 (2H,d, J=8.7 Hz); 7.22-7.24(1H,m); 7.50 (2H,d, J=8.7 Hz); 7.56(2H,d,J=8.7Hz); 7.80(2H,d J=8.7 Hz); 7.88(1H,d,J=5.0Hz); 8.04(1H d, J=3.3 Hz); 10.15(2H,broad); 10.34(1H,broad s).
4	C28H25N3O6 S	DCI + CH4 m/e MH+ (532,100)	d6-DMSO 1.60-1.80(4H,m); 2.30(2H,t,J=7.2Hz); 4.08(2H,t,J=6.1Hz); 6.74(1H,s); 6.74(1H,s); 6.98(2H d, J=8.7Hz); 7.21-7.23(1H,m); 7.52(2H, d,J=8.7Hz); 7.57(2H,d,J=8.7Hz); 7.80 (2H,d,J=8.7Hz); 7.87(1H, dd, J=0.8, 4.9Hz); 8.04(1H,dd,J=0.8,3.9Hz); 10.15 (2H,broad); 10.35(1H,s). OH not visible
5	C29H26N4O6 S	DCI + CH4 m/e MH+(559,100)	d6-DMSO 1.80-1.83(2H,m); 2.37(2H,t,J=7.4Hz); 3.32-3.33(2H,m); 3.58(3H,s); 6.76(1H,s); 6.79(1H,s); 7.20-7.22(1H,m); 7.57(2H,d,J=8.6Hz); 7.62(2H,d,J=8.3Hz); 7.80(2H,d,J=8.6Hz); 7.87-7.89(3H,m); 8.04(1H,d,J=3.9Hz); 8.51(1H,t,J=5.5Hz); 10.33(3H,broad).
6	C28H24N4O6 S	DCI +NH3 m/e MH+(545,100)	d6-DMSO 1.77-1.79(2H,m); 2.28(2H,t,J=7.4Hz); 2.50-2.52(2H,m); 6.78(1H,s); 6.80(1H,s); 7.21-7.24(1H,m); 7.57(2H,d,J=8.7Hz); 7.63(2H,d,J=8.3Hz); 7.80(2H,d,J=8.7Hz); 7.88-7.90(3H,m); 8.04(1H,d,J=3.4Hz); 8.52(1H,broad t, J=5.6Hz); 10.35(3H,broad).
7	C32H32N4O6 S	DCI + NH3 m/e MH+(601,22), MNH4+ (618,100)	d6-DMSO 1.30-1.33(4H,m); 1.45-1.65(4H,m); 2.25-2.35(4H,m); 3.58(3H,s); 6.73(1H,s); 6.74(1H,s); 7.20-7.22(1H,m); 7.50(2H,d,J=8.7Hz); 7.57(2H,d,J=8.7Hz); 7.64(2H,d,J=8.7Hz); 7.80(2H,d,J=8.7Hz); 7.88(1H,d,J=5.0Hz); 8.04(1H,d,J=3.5Hz); 10.0(1H,s); 10.18(2H,broad); 10.33(1H,s).
8	C34H36N4O6 S	DCI + NH3 m/e MH+(629,100), MNH_4+(646,60)	d6-DMSO 1.28-1.35(8H,m); 1.52-1.55(2H,m); 1.60-1.63(2H,m); 2.24-2.36(4H,m); 3.58(3H,s); 6.73(1H,s); 6.75(1H,s); 7.21-7.23(1H,m); 7.50(2H,d,J=8.7Hz); 7.57(2H,d,J=8.7Hz); 7.65(2H,d,J=8.7Hz); 7.80(2H,d,J=8.7Hz); 7.89(1H,d,J=4.5Hz); 8.04(1H,d,J=3.5Hz); 10.0(1H,s); 10.18(2H,broad); 10.33(1H,s).
9	C31H30N4O6 S	DCI + NH3 m/e MH+(587,100)	d6-dmso 1.30-1.33(4H,m); 1.50-1.52(2H,m); 1.60-1.63(2H,m); 2.20(2H,t,J=7.3Hz); 2.30(2H,t,J=7.3Hz);

			6.74(1H,s); 6.75(1H,s); 7.21-7.25(1H,m); 7.50(2H,d,J=8.7Hz); 7.55(2H,d,J=8.7Hz); 7.65(2H,d,J=8.7Hz); 7.80(2H,d,J=8.7Hz); 7.90(1H,d,J=3.3Hz); 8.10(1H,d,J=0.9Hz); 10.0(1H,s); 10.20(2H,broad s); 10.35(1H,s). OH not visible
10	C33H34N4O6 S	DCI + NH3 m/e MH+(615,100)	d6-DMSO 1.28-1.38(8H,m); 1.50-1.52(2H,m); 1.60-1.62(2H,m); 2.19(2H, t,J=7.3Hz); 2.31(2H,t,J=7.3Hz); 6.73(1H, s); 6.75(1H,s); 7.21-7.24(1H,m); 7.50(2H, d,J=8.7Hz); 7.57(2H,d,J=8.7Hz); 7.65(2H, d,J=8.7Hz); 7.80(2H,d,J=8.7Hz); 7.89(1H, d,J=0.9Hz); 8.04(1H,d,J=3.4Hz); 10.0(1H, s); 10.33(1H,s).Two NH and OH not visible
11	C41H48N4O9 S	ESI^+ MH+ 773	d6-DMSO; 10.47 (1H,br), 10.20(1H,br), 10.14(1H,br), 8.06(2H,d), 7.88(1H,d), 7.80(2H,d), 7.56(2H,d), 7.51(2H,d), 7.22(1H,m), 6.99(2H,d), 6.73(2H,2 overlapping singlets), 4.18(1H,m), 4.02(2H,t), 2.29(2H,t), 2.20(2H,t), 1.80-1.60(6H,m), 1.14(9H,s), 1.13(9H,s)
12	C33H32N4O9 S	ESI^+ M+ @ 665 (M-OH) @ 649	d6-DMSO 10.35(1H,br), 10.16(1H,br), 10.11(1H,br), 8.04(2H,d,2.1Hz), 7.86(1H,d,2.9Hz), 7.79(2H,d,7.1Hz), 7.55(2H,d,7.1Hz), 7.50(2H,d,7.3Hz), 7.22-7.20(1H,m), 6.96(2H,d,7.3Hz), 6.72(2H,s), 4.19-4.17(1H,m), 4.01(2H,t,3.2Hz), 2.25(2H,t,5.1Hz), 2.20(2H,t,4.3Hz), 1.88-1.87(1H,m), 1.75-1.60(5H,m). Two OH not visible
13	C27H29N3O5	ESI m/e MH+(476,100%)	(d6-DMSO) 1.28-1.32(4H,m); 1.53-1.57(4H,m); 2.28-2.32(4H,m); 3.58 (3H,s); 6.73(1H,s); 6.75(1H,s); 7.31-7.32 (1H,m); 7.40-7.42(2H,m); 7.50(2H,d, J= 8.7Hz); 7.54(2H,d,J=7.5Hz); 7.64 (2H d,J =8.7Hz); 10.0(1H,s); 10.15(2H,broad s).
14	C26H27N3O5	ESI m/e MH+(462,100%)	(d6-DMSO) 1.29-1.31(4H,m); 1.49-1.51(2H,m); 1.58-1.61(2H,m); 2.20(2H,t, J=7.3Hz); 2.31(2H,t,J=7.3Hz); 6.72(1H,s) 6.74(1H,s); 7.32-7.33(1H,m); 7.39-7.42 (2H,m); 7.50(2H,d,J=8.7Hz); 7.54(2H,d, J=7.5Hz); 7.64(2H,d,J=8.7Hz); 10.0(1H, s); 10.20(2H,broad s); 11.92(1H,broad s).
15	C29H27N3O7	DCI+(NH3) M+ 529	DMSO 10.35(1H, s), 10.12(2H, broad), 7.94(1H, d, J=1.03), 7.82(2H, d, J=8.70), 7.56 - 7.50(4H, m), 7.36-7.33(1H, m,), 6.97(2H, d, J=8.76Hz), 6.74(1H, s), 6.74(1H,s); 6.71(1H, d, J=2.53Hz), 4.02(2H, t, J-5.81Hz), 3.59 (3H,s); 2.39

			(2H, t, J=7.28Hz), 1.76 - 1.69(4H, m).
16	C27H25N3O7 S2	DCI-NH3 M-. 567	DMSO 10.35(1H, s), 9.98(2H, broad), 8.06-8.05(1H, m), 7.88-7.86(1H, m), 7.80(2H,d,J=8.72Hz), 7.56(2H,d, J=8.75Hz), 7.50(2H,d,J=8.82Hz); 7.24-7.22(1H,m), 6.98(2H, d, J=8.79Hz), 6.74(1H, s), 6.74(1H, s), 4.00(2H, t, J=5.93Hz), 2.46(2H, t, J=7.82); 1.80 - 1.69(4H, m). OH not visible
17	C28H25N3O7	DCI+NH3 MH+ 516, MHN4+ 533	DMSO 10.31(3H, broad), 7.94(1H, s), 7.82(2H, d, J=8.72Hz), 7.54(2H, d, J=8.74Hz), 7.51(2H, d,J =8.78Hz), 7.36(1H, d, J=1.00Hz), 6.98(2H, d, J=8.76Hz), 6.73(1H, d, J=1.86Hz), 6.72 - 6.71(2H, m), 4.01(2H, t, J=6.04Hz), 2.28(2H, t, J=7.34Hz), 1.76 - 1.71(2H, m), 1.68 - 1.63(2H, m). OH not visible
18	C32H33N3O6 S	DCI + NH3 m/e MH+(588,57%); base peak (296)	d6-DMSO 1.23-1.47(6H,m); 1.53-1.55(2H,m); 1.70-1.74 (2H,m); 2.30(2H, t,J=7.4Hz); 3.58(3H,s); 4.0(2H, t, J=6.5Hz); 6.74(1H,s); 6.74(1H,s); 6.97(2H,d,J=8.8Hz); 7.21-7.25(1H,m): 7.50(2H,d,J=8.8Hz); 7.57(2H,d,J=8.7Hz); 7.80(2H,d,J=8.7Hz); 7.88(1H,dd, J=0.8,4.8Hz); 8.05(1H, d,J=0.8Hz); 10.15(2H,broad); 10.33 (1H,s).
19	C31H31N3O6 S	DCI + NH3 m/e MH+(574,100%)	d6-DMSO 1.22-1.56(8H,m); 1.72-1.74(2H,m); 2.20(2H,t,J=7.3Hz); 4.0(2H,t,J=6.4Hz); 6.75(1H,s); 6.75(1H,s); 6.97(2H,d,J=8.8Hz); 7.21-7.22(1H,m); 7.50(2H,d,J=8.8Hz); 7.56(2H,d,J=8.7Hz); 7.80(2H,d,J=8.7Hz); 7.87(1H,d,J=1.0Hz); 8.04(1H, d,J=3.2Hz); 10.18(2H,broad); 10.33(1H,s). OH not visible
20	C31H31N7O4 S	ESI M-H- 596	d6-dmso 1.28-1.47 (6H, m), 1.65-1.70 (4H, m), 2.87 (2H, d, J=7.5), 3.98 (2H, t, J=6.4), 6.70 (2H, s), 6.97 (2H, d, J=8.8), 7.22 (1H, m), 7.51 (2H, d, J=8.9), 7.55 (2H, d, J=8.8), 7.78 (2H, d, J=8.7), 7.83 (1H, d, J=4.9), 8.03 (1H, d, J=2.9), 10.08 (1H, s), 10.12 (1H, s), 10.34 (1H, s)
21	C26H28N6O3	ESI MH+ 473	d6-DMSO 1.25-1.40 (6H, m), 1.62- 1.75 (4H, m), 2.82 (2H, t, J=7.5), 3.96 (2H, t, J=6.5), 6.72 (2H, d, J=5.8), 6.88 (2H, d, J=8.7), 7.28 (1H, t, J=7.3), 7.40 (2H, t, J=7.5), 7.48-7.58 (4H, m), 10.10-10.20 (2H, br.s)

CLAIMS

1. A compound which is a diketopiperazine of formula (I):



I

wherein X is selected from H and NHCOZ wherein Z is a thiophene or furan ring;

Y is selected from O, S, NHCO and CONH;

Q is a C_1-C_8 alkylene chain which is unsubstituted or substituted at any position along the chain by a group CO_2R^1 where R^1 is H or C_1-C_6 alkyl; and

R^2 is selected from:

a) CO_2R^1 wherein R^1 is as defined above;

b) tetrazole, which is bonded to Q via its carbon atom;

c) SO_3H;

and

d) $CONHR^3$ where NHR^3 derives from an amino acid R_3NH_2;

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim1 wherein, in formula (I):

X is NHCOZ wherein Z is thiophene;

Y is selected from O, NHCO and CONH;

Q is a C_3-C_8 alkylene chain which is unsubstituted or substituted at any position by a group CO_2R^1 as defined above; and

R^2 is (a) CO_2R^1 as defined above, (b) tetrazole bonded to Q via its carbon atom, or (c) $CONHR^3$ wherein R^3 is $-CH(COOH)CH_2CH_2COOH$.

3. A compound according to claim 1 wherein, in formula (I):

X is NHCOZ wherein Z is thiophen-2-yl;

Y is O or NHCO;

Q is C_3-C_8 alkylene which is unsubstituted or substituted at the carbon atom adjacent to Y by a group CO_2R^1 as defined above; and

R^2 is CO_2R^1 as defined above or is tetrazole.

4. A compound according to claim 1 which is selected from:

2-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-pentanedioic acid di-*tert*-butyl ester

2-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-pentanedioic acid

5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoic acid methyl ester

5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoic acid

4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-butyric acid methyl ester

4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-benzoylamino]-butyric acid

7-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid methyl ester

9-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-nonanoic acid methyl ester

7-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid

9-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-nonanoic acid

2-{5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoylamino}-pentanedioic acid di-*tert*-butyl ester

2-{5-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-pentanoylamino}-pentanedioic acid

7-[4-(5-Benzylidene-3,6-dioxo-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid methyl ester

7-[4-(5-Benzylidene-3,6-dioxo-piperazin-2-ylidenemethyl)-phenylcarbamoyl]-heptanoic acid

5-[4-(5-{4-[(Furan-2-carbonyl)-amino]-benzylidene}-3,6-dioxo-piperazin-2-ylidenemethyl)-phenoxy]-pentanoic acid methyl ester

4-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-butane-1-sulfonic acid

5-[4-(5-{4-[(Furan-2-carbonyl)-amino]-benzylidene}-3,6-dioxo-piperazin-2-ylidenemethyl)-phenoxy]pentanoic acid

8-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-octanoic acid methyl ester

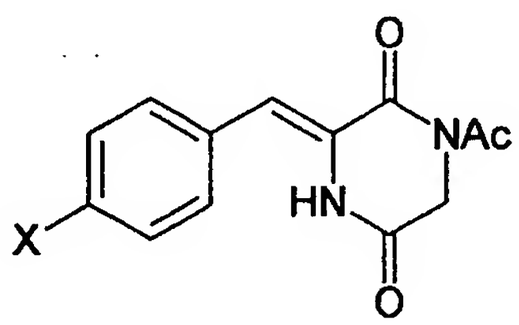
8-[4-(3,6-Dioxo-5-{4-[(thiophene-2-carbonyl)-amino]-benzylidene}-piperazin-2-ylidenemethyl)-phenoxy]-octanoic acid

Thiophene-2-carboxylic acid [4-(3,6-dioxo-5-{4-[7-(1*H*-tetrazol-5-yl)-heptyloxy]-benzylidene}-piperazin-2-ylidenemethyl)-phenyl]-amide

3-Benzylidene-6-{4-[7-(1*H*-tetrazol-5-yl)-heptyloxy]-benzylidene}-piperazine-2,5-dione

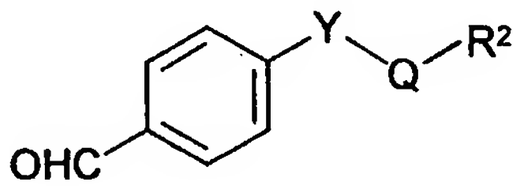
5. A process for producing a compound as defined in claim 1, which process comprises

(a) condensing a compound of formula (II):



(II)

wherein X is as defined is claim 1, with a compound of formula (III):



(III)

wherein Y, Q and R^2 are as defined in claim 1, in the presence of a base in an organic solvent.

6. A process according to claim 5 which further comprises converting a resulting diketopiperazine of formula (I) into another diketopiperazine of formula (I), and/or converting a resulting diketopiperazine of formula (I) into a pharmaceutically acceptable salt thereof.

7. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as an active principal, a compound as claimed in any one of claims 1 to 4.

8. A compound as defined in any one of claims 1 to 4 for use in a method of treatment of the human or animal body by therapy.

9. A compound as claimed in claim 8 for use as an inhibitor of PAI-1.

10. Use of a compound as claimed in any one of claims 1 to 4 in the manufacture of a medicament for use as an inhibitor of PAI-1.

11. Use according to claim 10 wherein the medicament is for use in treating a disease or disorder associated with elevated or inappropriate levels of PAI-1.

12. Use according to claim 10 or 11 wherein the medicament is for use in treating a haemostatic or thrombotic disorder.

INVESTOR IN PEOPLE

Application No: GB 0101225.1
Claims searched: 1-12

Examiner: Darren Handley
Date of search: 1 July 2002

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.T):

Int Cl (Ed.7): C07D 241/02, 307/36, 333/10, 403/10, 403/14, 405/10, 405/14, 409/10, 409/14

Other: Online: EPODOC, JAPIO, WPI; CAS-ONLINE

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	GB 2286395 A	(XENOVA) - see example 15, page 44 and compound 1910, page 7.	
X	GB 2284602 A	(XENOVA) - see page 11, line 35-page 14, line 14, compounds 87, 93, 96-97 & 101 in table 2 and reference examples 1 & 2 on pages 33-35.	1, 4-12
X	GB 2284420 A	(XENOVA) - see compounds 93, 96-97 & 101 in table 1, page 1, line 2- page 2, line 9 and page 13, line 4-page 14, line 9.	1, 4-12
A	US 5750530 A	(BRYANS) - see claim 3	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.